Research Paper

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Glyburide Actions on the Dihydropyridine-Sensitive Ca^{2+} Channel in Rat Vascular Muscle

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Vasodilation
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Ca^{2+} influx
Ba^{2+} currents
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Ca^{2+} agonist
Ca^{2+} antagonist

Abstract

The effects of glyburide, a purportedly selective ATP-sensitive K^+ channel antagonist, were studied on dihydropyridine (DHP)-sensitive (L-type) Ca^{2+} channel currents in rat aortic muscle cells. Whole-cell voltage-clamp Ba^{2+} currents (I_{Ba}) were recorded at a series of test potentials (V_T) from −30 to +60 mV during 300-ms voltage steps from a holding potential of −80 mV. Bay k8644 (1 μM) increased peak divalent cation currents from 47.2 ± 15.1 to 102.6 ± 13.4 pA, and the current-voltage relationship curve was shifted 10 mV to the left (n = 5). The combination of 10 μM glyburide with 1 μM Bay k8644 further increased Bay k8644-enhanced I_{Ba} in each cell (average of 223.7 ± 26.4 pA, n = 5), and caused a further 10 mV hyperpolarizing (leftward) shift of the activation curve. The kinetics of I_{Ba} were also changed (more rapid inactivation) by glyburide. These stimulatory actions of glyburide were reversed on washout. In contrast to this apparent synergism with Bay k8644, 10 μM glyburide alone inhibited (rather than potentiated) I_{Ba} by about 20% at V_T of 0, +10, and +30 mV. Increasing glyburide concentration to 30 μM further inhibited the I_{Ba} to about 40–50% of controls. With the pure agonist isomer, 0.5 μM Bay R5417, at theoretically the same concentration of the minus enantiomer as is present in Bay k8644, I_{Ba} increased from 137 ± 18.3 pA to 354.2 ± 12.4 pA (n = 4). However, the combination of 10 μM glyburide and 0.5 μM Bay R5417 failed to further enhance I_{Ba} (tendency to block) at any V_T, implying that the glyburide-Bay k8644 interaction may depend on both the stronger agonist and weaker antagonist isomers. I_{Ba} enhanced by 10 μM glyburide seem to flow through L-type Ca^{2+} channels because they were significantly blocked by the DHP antagonists, nimodipine and nisoldipine. Nimodipine (3 μM) inhibited the peak I_{Ba} by 30%, and 3 μM nisoldipine blocked I_{Ba} by almost 70%. Because glyburide produced opposite effects (block) when combined with the pure agonist enantiomer of Bay k8644, similar to the glyburide alone, but opposite to enhancement of racemic Bay k8644 agonist action, it is likely that there are multiple sites for modulation of vascular muscle L-type Ca^{2+} channels. Possible explanations include the existence of subtypes of DHP receptors, and glyburide actions that depend on the balance of DHP agonist versus antagonist influence. Glyburide could either be weakening the Ca^{2+} antagonist actions or acting at a separate sulfonylurea site that interacts with DHP sites.

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Introduction

The high threshold, voltage-dependent, dihydropyridine (DHP)-sensitive Ca^{2+} channel (L-type) plays an important role in the regulation of a variety of cellular functions, such as membrane excitability, muscle contraction, synaptic transmission, and other forms of secretion [1]. Evidence from studies using molecular biological approaches has supported the suggestion from functional reports of a diversity of Ca^{2+} channels in different cells and under different conditions [2]. Both L-type and non-L-type Ca^{2+} channels can be arranged in a family diagram based on homologies [3]. Increasingly, the L-type Ca^{2+} channel has been appreciated in the role of the voltage sensor, at least in the skeletal muscle type of excitation-contraction coupling (E-C coupling) [4, 5].

Increases in intracellular Ca^{2+} concentrations due to influx through these Ca^{2+} channels or to release from storage sites might importantly influence other ion movements, such as Ca^{2+}-dependent Cl^- current or Ca^{2+}-dependent K^+ current [6–8]. These Ca^{2+}-dependent K^+ or Cl^- currents would limit depolarization. On the other hand, increases in outward K^+ currents via K^+ channel activators result in hyperpolarization of the cell membrane, thus reducing influx of Ca^{2+} through voltage-dependent Ca^{2+} channels. And the resulting decrease of intracellular Ca^{2+} results in relaxation of the muscle fibers. However, the mechanisms of action of neither K^+ channel activators nor blockers have yet been fully established.

Previous studies with ultra-high-sensitivity digital imaging microscopy [9] showed that, in addition to increasing K^+ conductance, pinacidil and cromakalim cause intracellular Ca^{2+} redistribution which may contribute to the membrane potential-independent part of the vasorelaxant action of the K^+ channel openers. Data from dog coronary artery [10], dog mesenteric artery [11], and rat aorta [12] also suggested that for pinacidil, there is a second mechanism which does not involve hyperpolarization, but inhibits Ca^{2+} influx. On the other hand, contribution of an influx of extracellular Ca^{2+} (via Ca^{2+} channels) in vasoconstrictor responses to K^+ channel blockers, such as tetraethylammonium and aminopyridine, have been demonstrated [13, 14].

There has been great interest in one particular K^+ channel blocker recently. This purportedly selective ATP-sensitive K^+ channel antagonist, glyburide [15–17], was investigated for the possibility of either increasing or decreasing vascular muscle cell (VMC) Ca^{2+} currents. We found both kinds of actions, directly on VMC Ca^{2+} channels but dependent on simultaneous DHP exposure. These interactions of glyburide with the L-type Ca^{2+} channel agonists (Bay k8644 and Bay R5417) and antagonists (nimodipine and nisoldipine) are consistent with multiple modulation sites on VMC L-type Ca^{2+} channels.

Methods

Cell Culture

Primary cultures of aortic VMC were prepared from normotensive N/Nih control rats from a colony maintained by our laboratory. Aortas of neonatal (1–3 days old) rats were excised and put in MEM solution. After a rinse in MEM, the tissue was soaked for 10 min in KG solution. The aorta was then minced with fine dissecting scissors. Aorta fragments were incubated at 37°C with collagenase (30 mg in 10 ml KG solution containing 0.1 μM Ca^{2+}) for 30 min. The collagenase supernatant was discarded and the sediment was exposed to 3 or 4 15-min incubations in 15 ml of 1 mg/ml trypsin in KG solution. After each incubation, the supernatant was removed and placed in 10 ml horse serum on ice. The combined supernatant fractions were then centrifuged at 200 *g* for 5 min. The cell pellets were resuspended in 10 ml of CV3M plus 5 μM 5-bromo-2′deoxyuridine (BrdU) and sedimented in a 15-ml tissue culture flask [18]. Nonattached cells were resuspendend in CV3M + 5 μM BrdU, diluted to a density of 70,000 cells/ml, and plated on poly-*L*-lysine-coated 9 × 22 mm glass coverslips. Cells were kept in a 5% CO_2 and 95% air incubator at 95% humidity and 37°C, and used after 2–3 days, during which spontaneous contractions, membrane excitability, and high sensitivity to norepinephrine were maintained [18]. The conditions were optimized to provide the highest quality of undamaged VMC [19], and thus are at the opposite pole from subcultured cells, which we deliberately avoided. Additonal arguments in support of our cell culture approach to VMC function can be found in Hermsmeyer et al. [20].

Electrophysiological Measurements

For voltage-clamp analysis, a glass coverslip with attached rat aorta cells was transferred to the laminar flow chamber [21] on the stage of a Zeiss Axiovert microscope. The flow chamber was perfused with ionic solution continuously. All experiments were performed at room temperature (23°C).

Tight seal pipette electrodes were prepared from thin wall 7052 glass tubing (A-M Systems, Inc.) by using a Brown-Flaming micropipette puller (model P-87, Sutter Instruments Co.) with a five-stage program. Pipette tips were heat polished and filled with the pipette solution. Whole cell recordings of Ca^{2+} channel currents were made using 3–4 MΩ pipettes. The recording pipette was connected to the input stage of the CV-3 high-speed, low-noise, current-to-voltage converter fixed to the head of a three dimensional hydraulic manipulator (Narishige Scientific Co.). After formation of a gigaohm seal (5–10 GΩ), membrane holding potential (V_H) was set to –45 mV in voltage-clamp mode, and rupture of the membrane patch was achieved by α-toxin pore formation and/or application of gentle suction through the pipette, as indicated by increased capacitive transient and current noise [22].

Experiments were performed using an Axopatch 1B amplifier (Axon Instruments), Bessel filtered at 1,000 Hz. Ca^{2+} channel currents were elicited by 300-ms depolarizations at intervals of 6 s. The

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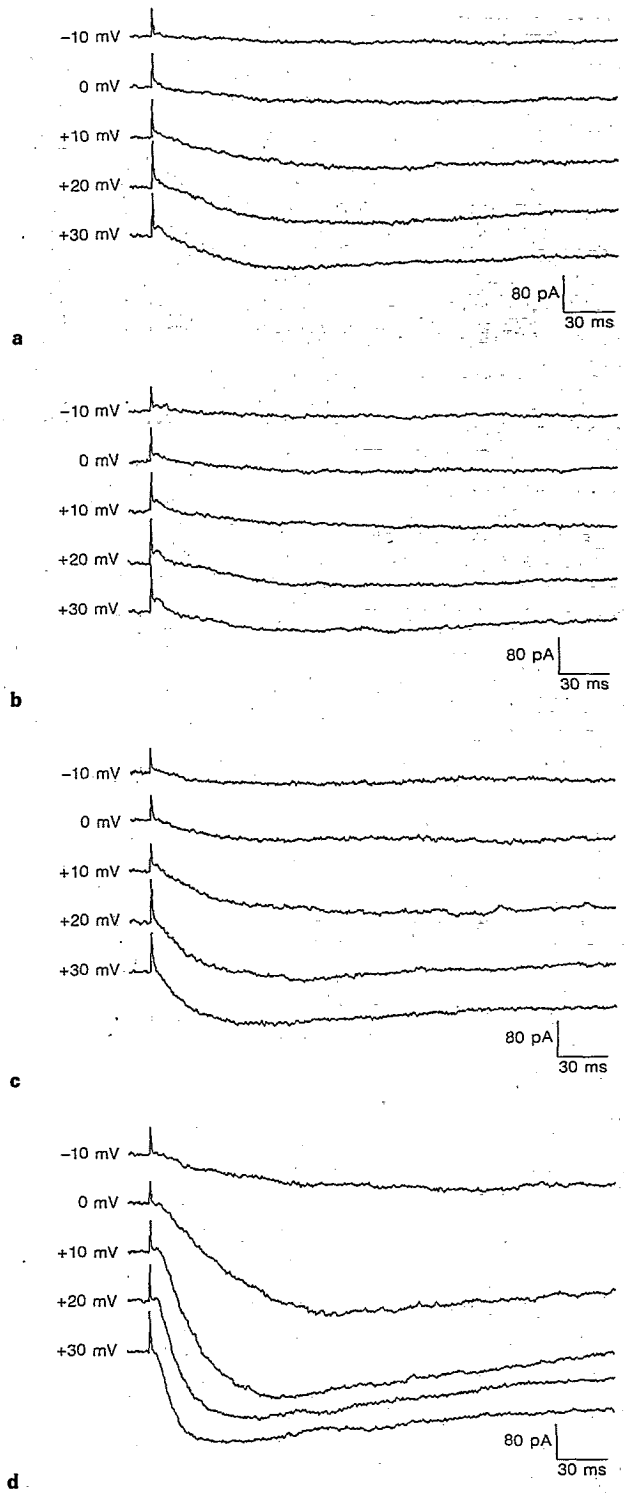

amplifier output was monitored on a two-channel digital storage oscilloscope (Tektronix 2424L), and the data were digitized and stored on floppy disks to permit further analysis using a TL-1 A-D interface at a sampling frequency of 10 kHz, with pClamp software (Axon Instruments) running on a Hewlett Packard Vectra ES computer.

The first three test potential (V_T) steps to +10 mV from a V_H of −80 mV were used to assess Ca^{2+} channel acceptability, and only cells with ≤10% rundown during controls were used for the data herein. The compensation of leakage and capacitance current was carried out by subtraction of the average of four 10-mV hyperpolarizing steps just before each depolarizing episode. These currents were appropriately scaled to currents during depolarizing steps. No data are included from cells which required a correction of leak current by more than a few percent, as leaky cells were excluded. The compensation procedure primarily served to reduce capacitance artifacts. Cell capacitance averaged about 30 pF and cell input resistance averaged 3 GΩ (τ = 900 μs) for these VMC, consistent with a physical volume of 0.6 pl, as observed during the studies.

Solutions and Drugs

The composition of the CV3M solution consisted of: 4.0 m*M* *L*-glutamine, 20 μg/ml gentamicin, 20 m*M* HEPES (pH = 7.3), and 16 m*M* $NaHCO_3$ dissolved in 85% MEM-Earle salts, 15% horse serum. The KG solution consisted of (in m*M*): 140 K-glutamate, 25 HEPES (pH = 7.3), 16 NaH_2PO_4, 16.5 dextrose, and 0.014 phenol red.

For voltage-clamp analysis of divalent ion currents, the perfusion chamber contained a Ba20TEA (external) solution which consisted of (in m*M*): 20 $BaCl_2$, 120 TEA-Cl, 1 $MgCl_2$, 5.5 dextrose, 15 HEPES (pH = 7.4), with the pH adjusted to 7.4 by the addition of TEAOH. The Ba20TEA + Bay k8644 or Ba20TEA + Bay R5417 solutions were exactly the same as the Ba20TEA solution except that 1 μ*M* Bay k8644 or 0.5 μ*M* Bay R5417 were added, respectively. The test solutions and glyburide perfused the chamber continuously at a constant flow rate of 0.6 ml/min. The pipette solution consisted of (in m*M*): 140 Cs Glutamate, 1 $MgCl_2$, 10 HEPES, 3.4 EGTA, 0.5 ATP dipotassium salt, 0.5 GTP Tris salt, and pH adjusted to 7.4 with CsOH. To gain access to the cell interior without suction, permeabilization of the membrane patch in the tight seal was carried out by adding 200 U/ml α-toxin to the pipette solution.

Nimodipine and nisoldipine, supplied by Miles, Inc., West Haven, Conn., USA, were dissolved in 50% PEG/DMSO (v/v) to make 1 m*M* stock solutions. Bay k8644 and Bay R5417, also supplied by Miles, Inc., were dissolved in 50% PEG/DMSO (v/v) to make

Fig. 1. Effects of Bay k8644 and glyburide on the Ca^{2+} channel current recorded from a rat aorta vascular muscle cell (VMC). I_{Ba} recorded under control conditions (**a**) and in 10 μ*M* glyburide (without Bay k8644) (**b**) in the perfusion solution showed slight inhibition of I_{Ba}; application of 1 μ*M* Bay k8644 significantly potentiated I_{Ba} (**c**); and the combination of 1 μ*M* Bay k8644 and 10 μ*M* glyburide further enhanced VMC I_{Ba} (**d**). Step test pulses (V_T) were applied over the voltage range of −30 to +60 mV from a holding potential (V_H) of −80 mV. Bath solution contained 20 m*M* Ba^{2+} as the charge carrier. Pipette solution contained cesium glutamate, 1 m*M* Mg^{2+}, 50 n*M* Ca^{2+} in EGTA buffer, 0.5 m*M* ATP, and 0.5 m*M* GTP.

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10-mM stock solutions. Glyburide (Sigma) was dissolved in DMSO to make a 10-mM stock solution. Aliquots of these stock solutions were added to the Ba20TEA solution to obtain the desired final concentrations.

Data Analysis

Data are given as mean ± SEM. Statistical significance was determined by Student's unpaired t test and Tukey's method for one-way analysis of variance. Values of $p < 0.05$ were considered statistically significant.

Results

Currents were recorded at test potentials (V_T) of −30 to +60 mV from a holding potential (V_H) of −80 mV in solutions designed to isolate divalent cation currents (20 mM Ba^{2+} in the extracellular perfusion solution). Figure 1 shows a typical pattern of Ba^{2+} currents (I_{Ba}) recorded from an aortic vascular muscle cell (VMC). Depolarization to potentials less negative than −20 mV elicited little or no I_{Ba}. I_{Ba} began to be evident positive to about −10 mV, and peak I_{Ba} occurred at about +30 mV (fig. 2). These voltage-dependent currents decayed slowly, with a half-life of ≥ 300 ms.

The 1,4-DHP derivative, Bay k8644 (1 μM), produced a significant increase in the size of I_{Ba} from 47.2 ± 15.1 pA to 102.6 ± 13.4 pA (n = 5). The largest I_{Ba} reached over 140 pA at +20 mV (fig. 1, 2). Bay k8644-induced enhancement of peak I_{Ba} was largest at weak depolarizations, and became progressively smaller at more positive potentials. The maximum increase by Bay k8644 was to more than twice the peak amplitude of control value, and occurred at about +20 mV (fig. 1, 2).

Addition of glyburide (10 μM) to the perfusion solution (which already contained 1 μM Bay k8644) further enhanced I_{Ba}. Glyburide altered both the voltage and kinetics of I_{Ba} through the Ca^{2+} channels. As with enhancement by Bay k8644, increases in I_{Ba} were markedly greater for small depolarizations than for larger, more positive V_T. The maximum increase was to ~3-fold the amplitude in the control, which was observed at V_T = 0 mV. The voltage which produced the peak I_{Ba} (223.7 ± 36.4 pA, n = 5) was shifted from +20 to +10 mV (about 10–15 mV) by glyburide (fig. 1–4). Both amplitudes at peak I_{Ba} and at 200 ms were similarly enhanced by glyburide at all V_T (fig. 2). Current-voltage plots show that glyburide caused a further hyperpolarizing (leftward) shift of the activation curve by 10–15 mV beyond that produced by Bay k8644. I_{Ba} decayed more rapidly in the presence of 10 μM glyburide with more pronounced action as the depolarizing pulse grew stronger (fig. 1). Increasing the concentration of glyburide to 30 μM caused a greater increase in I_{Ba} (fig. 3a, 4a). Lower concentrations of glyburide (3 μM) did not increase I_{Ba} at any voltage.

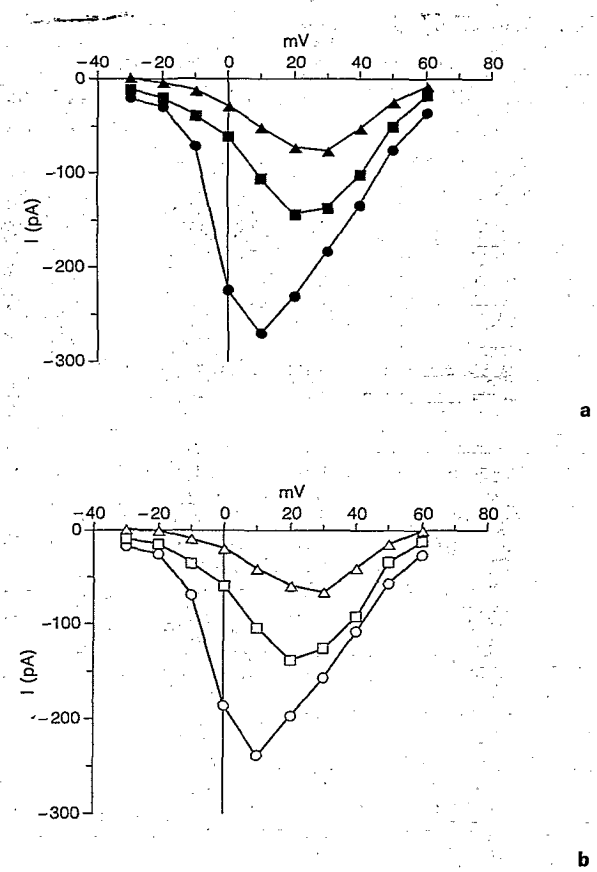

Fig. 2. Effects of Bay k8644 and glyburide on VMC Ca^{2+} channel currents. Peak current amplitudes (**a**; filled symbols) and I_{Ba} amplitudes measured at 200 ms (**b**; open symbols) are shown. I_{Ba} amplitudes measured under control condition (triangles), with 1 μM Bay k8644 (squares), and with the combination of 1 μM Bay k8644 and 10 μM glyburide (circles) are plotted against V_T. Data are from the same VMC as figure 1.

Glyburide-increased I_{Ba} were reversed on washout for 3–5 min and significantly blocked by DHP Ca^{2+} antagonists, nimodipine and nisoldipine. As illustrated in figure 5, I_{Ba} (enhanced by 10 μM glyburide plus 1 μM Bay k8644) were diminished significantly by 3 μM nimodipine and more strongly by 3 μM nisoldipine. While 3 μM nimodipine attenuated the I_{Ba} (which occurred at

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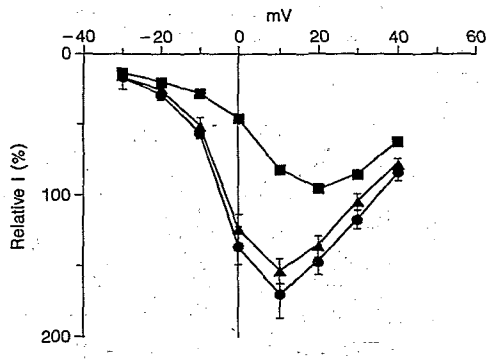

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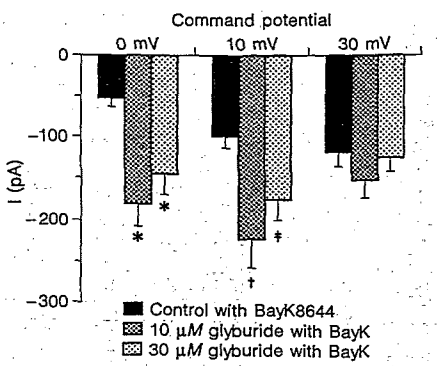

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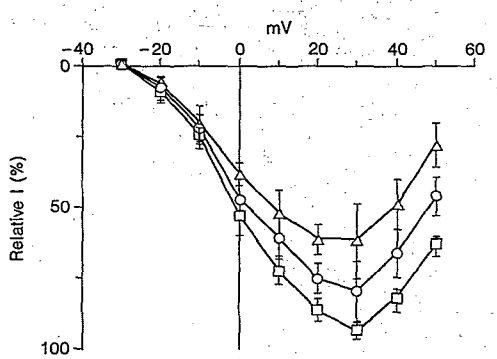

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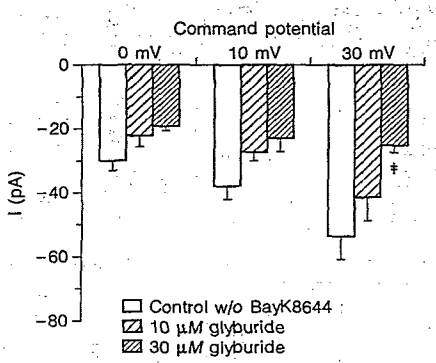

b

Fig. 3. Comparison of the effects of glyburide in combination with Bay k8644 (**a**) or alone (**b**) on aortic cell I_{Ba}. In **a**, normalized Ca^{2+} channel currents recorded from application of 1 μ*M* Bay k8644 (squares), combination of 1 μ*M* Bay k8644 and 10 μ*M* glyburide (circles), and combination of 1 μ*M* Bay k8644 and 30 μ*M* glyburide (triangles) show the leftward shift by glyburide. I_{Ba} were normalized to values at peak inward current (81.0 ± 15.1 pA; n = 10) recorded from application of 1 μ*M* Bay k8644. In **b**, normalized I_{Ba} recorded from control (squares), 10 μ*M* glyburide treatment (circles), and 30 μ*M* glyburide treatment (triangles) show that without Bay k8644, glyburide significantly inhibited Ca^{2+} channel currents. I_{Ba} were normalized to values at peak inward currents (45.1 ± 12.6 pA; n = 7) recorded from controls.

Fig. 4. Actions of 10 μ*M* and 30 μ*M* glyburide on aortic VMC I_{Ba} with (**a**) or without (**b**) Bay k8644. Currents were evoked by V_T of 0 mV, +10 mV, and +30 mV from a V_H of −80 mV. In **a**, I_{Ba} were recorded during application of 1 μ*M* Bay k8644 in Ba20 TEA perfusion solution (n = 5). In **b**, I_{Ba} were recorded from perfusion of Ba20 TEA solution without Bay k8644 (n = 4). Significant differences from the control for the same group at the same V_T are indicated: * $p < 0.01$, † $p < 0.02$, and ‡ $p < 0.05$. Vertical bars represent ± SEM.

+20 mV) by 30%, 3 μ*M* nisoldipine reduced I_{Ba} by almost 70%.

Surprisingly, without Bay k8644 (1 μ*M*) in the perfusion solution, glyburide blocked rather than enhanced I_{Ba} and caused no voltage shift. Aortic VMC I_{Ba} in 10 μ*M* glyburide were inhibited (by about 20% at V_T of 0, +10, or +30 mV), rather than potentiated. Further increasing the glyburide concentration to 30 μ*M* inhibited I_{Ba} significantly more than 10 μ*M*. The block of I_{Ba} by glyburide was about 40–50%, with greatest inhibition at the most positive (+30 mV) V_T (fig. 3b, 4b), and partial return toward control amplitudes on washout.

As shown in figures 1 and 2, VMC I_{Ba} were enhanced when exposed to DHP agonist, and further enhanced by

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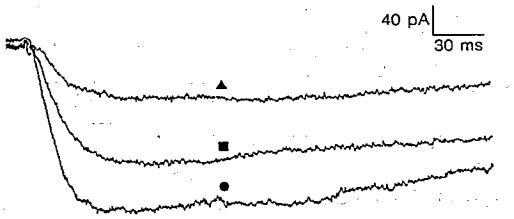

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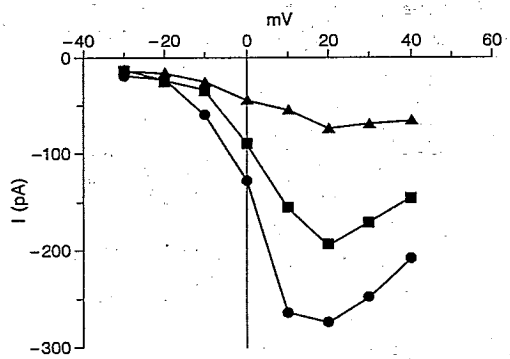

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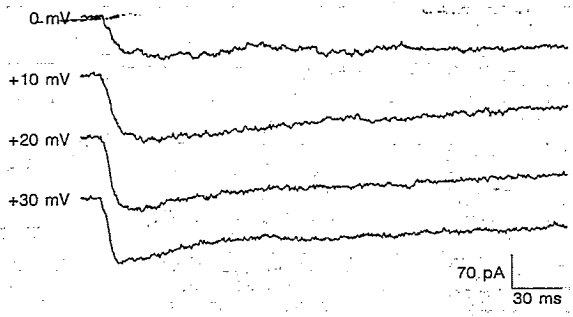

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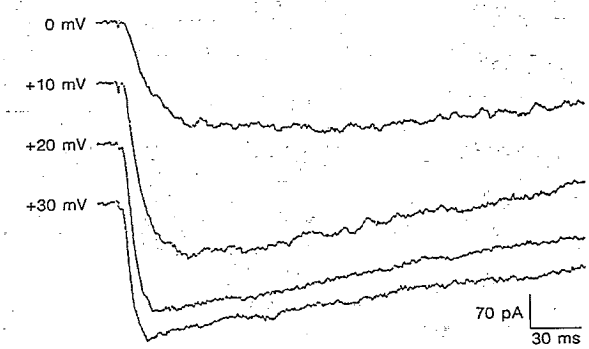

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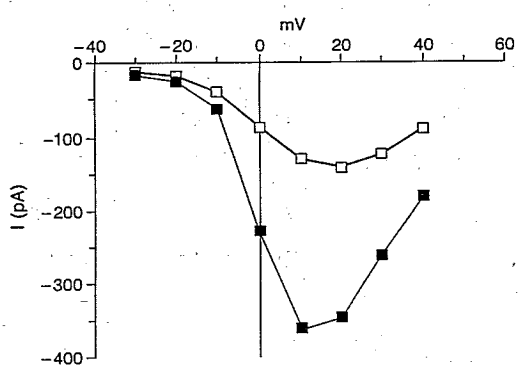

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Fig. 5. Effects of nimodipine and nisoldipine on Bay k8644 and glyburide-enhanced VMC Ca^{2+} channel currents (**a**) and peak I_{Ba} values (**b**). I_{Ba} were recorded from the combination of 0.1 μ*M* Bay k8644 and 10 μ*M* glyburide (circles), the combination of 0.1 μ*M* Bay k8644, 10 μ*M* glyburide and 3 μ*M* nimodipine (squares), and the combination of 0.1 μ*M* Bay k8644, 10 μ*M* glyburide, and 3 μ*M* nisoldipine (triangles). I_{Ba} were evoked by a V_T of +20 mV from a V_H of −80 mV (**a**) and from −80 mV to the V_T (abcissa) in (**b**).

Fig. 6. Effects of Bay k8644 (**a**) and Bay R5417 (**b**) on Ca^{2+} channel currents and peak I_{Ba} amplitudes (**c**). I_{Ba} were increased by application of 1 μ*M* Bay k8644 and 0.5 μ*M* Bay R5417. I_{Ba} were evoked by V_T (−30 mV to +40 mV) from a V_H of −80 mV. Peak I_{Ba} amplitudes measured with treatment of 1 μ*M* Bay k8644 (open symbols) were significantly less than those in 0.5 μ*M* Bay R5417 (filled symbols), which are plotted against V_T (**c**).

glyburide. This cooperative glyburide-Bay k8644 augmentation of I_{Ba} was dependent on the presence of the Ca^{2+} channel agonist, Bay k8644 (1 μ*M*), since glyburide decreased I_{Ba} when acting alone (fig. 1–4). To study the mechanism of the unexpectedly complicated actions of glyburide on Ca^{2+} channels in more detail, we used Bay R5417 (the minus isomer of Bay k8644), and investigated whether there is stereoisomer dependence of the cooperative interaction of glyburide and DHP. To approximately match the concentration of minus enantiomer that would be found in 1 μ*M* Bay k8644, 0.5 μ*M* Bay R5417 was used.

Switching perfusion solution from 1 μ*M* Bay k8644 to 0.5 μ*M* Bay R5417 further increased I_{Ba} (fig. 6), possibly

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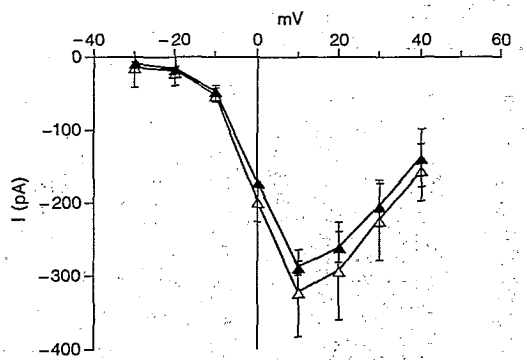

Fig. 7. Actions of 0.5 μM Bay R5417 (open symbols) and combination of 0.5 μM Bay R5417 and 10 μM glyburide (filled symbols) on aortic VMC I_{Ba} (n = 4). Currents were evoked by V_T of −30 mV to +40 mV from a V_H of −80 mV. Combining glyburide and Bay R5417 failed to further enhance the currents at all V_T, possibly because the currents were already at a maximum with Bay R5417.

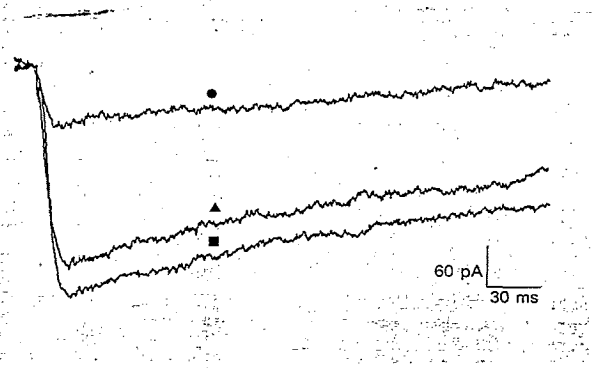

Fig. 8. Actions of glyburide combined with nisoldipine on Bay R5417 enhanced VMC Ca^{2+} channel currents. I_{Ba} were recorded in 0.5 μM Bay R5417 (square), the combination of 10 μM glyburide and 0.5 μM Bay R5417 (triangle), and the combination of 10 μM glyburide, 0.5 μM Bay R5417, and 3 μM nisoldipine (circle). Currents were evoked by a V_T of +20 from a V_H of −80 mV. On these I_{Ba} enhanced by Bay R5417, glyburide had only a small inhibitory effect, while nisoldipine effectively inhibited I_{Ba}.

by eliminating the weaker but still significant blocking action of +Bay k8644. Peak I_{Ba} at +10 mV were increased from 137.6 ± 19.3 pA to 354.2 ± 12.4 pA (n = 4). Comparing current-voltage plots (fig. 6 vs. 2), 0.5 μM Bay R5417 left-shifted the activation curve and enhanced I_{Ba}. However, addition of glyburide (10 μM) to the perfusion solution (with 0.5 μM Bay R5417) failed to further enhance I_{Ba} (and tended to inhibit) at any V_T (fig. 7). I_{Ba} enhanced by Bay R5417 with or without glyburide were significantly inhibited by 3 μM nisoldipine (fig. 8). The fraction of I_{Ba} blocked by 3 μM nisoldipine was the same as in the Bay k8644 (1 μM) and glyburide (10 μM) conditions, as if maximally enhanced currents were equivalently blocked by nisoldipine.

Discussion

In the present study, 10 and 30 μM glyburide, in the presence of 1 μM Bay k8644, dramatically enhanced Ca^{2+} channel currents in rat aortic VMC. Besides enhancing amplitude of I_{Ba}, glyburide shifted the current-voltage relationship curve in the negative direction 10–15 mV and tended to accelerate the decay of Ba^{2+} currents. The DHP Ca^{2+} agonist, Bay k8644, which increases cardiac contractility, enhances divalent cation influx of VMC as well as cardiac muscle cells [23–25] (fig. 1, 2). Bay k8644 altered the voltage- and time-dependence of Ca^{2+} channel currents, which suggests that Ca^{2+} channel gating is being modified [26]. Glyburide further enhanced both voltage- and time-dependence actions on the Ca^{2+} channel currents by Bay k8644. Effects of glyburide on Ca^{2+} channels in VMC were thus apparently cooperative with Bay k8644. Bay k8644 has been reported to bind specifically only to a DHP receptor, but not to other known receptors, e.g. dopaminergic, muscarinic, α- and β-adrenergic, serotonergic, histaminergic, γ-aminobutyric acid (GABA), diazepam, or opiate receptors [23]. Thus, Ca^{2+} channel currents increased by glyburide most likely result from an interaction with the Bay k8644 site on the Ca^{2+} channel.

It has been shown that glyburide is one of the most potent inhibitors of ATP-sensitive K^+ channels in several vascular and uterine muscle preparations [9, 27–32]. Glyburide potently stimulates release of insulin from pancreatic acinar cells by blocking an ATP-sensitive K^+ channel [33, 34]. Whether the same mechanisms of action are found in both muscle contraction and insulin release is not known.

Glyburide reduced the opening of ATP-activated K^+ channels and induced insulin secretion in isolated pancreas at low concentrations (50–500 nM) [35, 36], whereas the concentrations of glyburide required (1–10 μM) to antagonize the vasodilator actions of the K^+ openers [28, 29, 32, 37] are 1–2 orders of magnitude higher. One possi-

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ble explanation is that pancreatic β-cell channels could have a 'sulphonylurea recognition site' that differs from that in VMC.

In dog coronary artery, glyburide (1–30 μ*M*) caused a dose-dependent reduction in both spontaneous isometric force and contractions induced by U46619, a thromboxane A_2-mimetic [38]. Glyburide (10 μ*M*) also significantly attenuated I_{Ba} in dog aortic VMC [39]. In the present study, agonist I_{Ba} enhancement actions of glyburide on rat aortic VMC Ca^{2+} channels were cooperative with Bay k8644, at concentrations (10–30 μ*M*) similar to those reported to block K^+ channels in vascular muscle [27, 29, 31, 40], and could thus also contribute to contraction.

Treatment with glyburide (10 or 30 μ*M*) alone inhibited Ca^{2+} channel currents (fig. 3b, 4b) in the present study. Though glyburide had no effect on resting tension in the rat aorta, glyburide could significantly relax prostaglandin (PG) $F_{2\alpha}$-induced contractions [40]. It was also reported by the same group that glyburide relaxed dog femoral, mesenteric, renal, coronary, and cerebral artery contractions produced by $PGF_{2\alpha}$. The enhanced block of I_{Ba} by glyburide at more positive V_T might suggest a possible Ca^{2+} channel antagonist action of glyburide that would contribute to relaxation. Thus, depending on conditions, glyburide might either increase or decrease contraction.

We might ask whether glyburide can act as a partial agonist as the logical counterpart to the partial (weak) antagonist effects of Bay k8644. Nitrendipine, an antagonist, shows a net inhibitory effect that is the result of both weak agonist and stronger antagonist effects [41]. The DHP Ca^{2+} antagonist, nifedipine, similarly produced both positive and negative inotropic effects in papillary muscle preparations at very low concentrations and higher concentrations, respectively [23, 42].

Bay R5417 (0.5 μ*M*), which is the active (–)enantiomer of Bay k8644, enhanced peak inward currents more thant the combination of glyburide and Bay k8644 (354.2 ± 23.5 pA, n = 4 vs. 223.7 ± 36.4, n = 5). The combination of glyburide and Bay R5417 did not further increase the currents. One possiblity to explain this phenomenon would be that (–)isomer alone produces the maximum increase of the open times and thus amplitudes of DHP-sensitive Ca^{2+} channel currents. Single-channel analysis shows that the primary effect of 1 μ*M* Bay k8644 is a large increase in the average open times of the Ca^{2+} channel [43, 44]. In addition, the (+)enantiomer of Bay k8644 is a weak Ca^{2+} antagonist that probably interferes with the agonist action of the (–)enantiomer, apparently at a site where glyburide can block or displace the antagonist action. Glyburide could be acting at either allosteric or interactive sites, e.g. the two DHP receptor binding sites. Alternatively, different subtypes of DHP receptors may exist, allowing activation or inhibition of the different subtypes of Ca^{2+} channel activity in response to glyburide, depending on the state of the different DHP receptors.

In conclusion, we studied the effects of glyburide on Ca^{2+} channel currents, and glyburide interactions with Ca^{2+} agonists (Bay k8644 and Bay R5417) and Ca^{2+} antagonists (nisoldipine and nimodipine) in rat aortic VMC. Our results show that glyburide produced significant effects that were opposite, depending on whether or not DHP agonist was present. These data suggest cooperative interactions of glyburide and DHP sites on the L-type Ca^{2+} channel and multiple modulation of DHP Ca^{2+} channels, with glyburide enhancing the agonist action of Bay k8644. Our study suggests that actions of glyburide on Ca^{2+} channels, in addition to K^+ channel block, should be considered as important.

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